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Research report

SB 239063, a novel p38 inhibitor, attenuates early neuronal injury following ischemia

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Abstract

The aim of the present study was to evaluate p38 MAPK activation following focal stroke and determine whether SB 239063, a novel second generation p38 inhibitor, would directly attenuate early neuronal injury. Following permanent middle cerebral artery occlusion (MCAO), brains were dissected into ischemic and non-ischemic cortices and Western blots were employed to measure p38 MAPK activation. Neurologic deficit and MR imaging were utilized at various time points following MCAO to monitor the development and resolution of brain injury. Following MCAO, there was an early (15 min) activation of p38 MAPK (2.3-fold) which remained elevated up to 1 h (1.8-fold) post injury compared to non-ischemic and sham operated tissue. Oral SB 239063 (5, 15, 30, 60 mg/kg) administered to each animal 1 h pre- and 6 h post MCAO provided significant ($P<0.05$) dose-related neuroprotection reducing infarct size by 42, 48, 29 and 14%, respectively. The most effective dose (15 mg/kg) was further evaluated in detail and SB 239063 significantly ($P<0.05$) reduced neurologic deficit and infarct size by at least 30% from 24 h through at least 1 week. Early (i.e. observed within 2 h) reductions in diffusion weighted imaging (DWI) intensity following treatment with SB 239063 correlated ($r=0.74$, $P<0.01$) to neuroprotection seen up to 7 days post stroke. Since increased protein levels for various pro-inflammatory cytokines cannot be detected prior to 2 h in this stroke model, the early improvements due to p38 inhibition, observed using DWI, demonstrate that p38 inhibition can be neuroprotective through direct effects on ischemic brain cells, in addition to effects on inflammation.

Theme: Disorders of the nervous system

Topic: Ischemia

Keywords: Stroke; p38 MAPK; Neuronal Injury; Neuroprotection; Focal ischemia

1. Introduction

The mitogen activated protein kinase (MAPK) family is composed of three main groups of kinases. Included among these kinases are the p42/44 extracellular-signal regulating kinase (ERK), c-Jun N-terminal kinase (JNK), and the p38 kinases [28,37]. The MAPK can be further divided into two major subfamilies; those regulated by growth factors promoting survival (ERK) and the stress activated protein kinases (SAPKs) which regulate apoptosis and inflammatory cell death [27,29,31,42].

Activation of the p38 MAPK pathway (phosphorylated p38 MAPK) has been implicated in playing a role in the regulation of pro-inflammatory cytokines and apoptosis [27,30,31,42]. In particular, in vitro studies demonstrated that monocytes require activation of p38 MAPK for LPS induced cytokine release. Inhibition of the p38 MAPK pathway using SB 203580 (specific for p38α and p38β) resulted in an inhibition of cytokine release from stimulated monocytes [26]. Several studies have demonstrated that p38 MAPK may regulate the transcription of cytokine mRNA [2] and/or possibly the translation of cytokines by

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inhibiting the critical step of phosphorylation of an AU-rich (AUUUA) repeat in the 3′ end of mRNA [25]. SB 203580 also was effective at reducing TNFα, IL-1β production [25,29,30,39,43] as well as the expression of several other inflammatory cytokines, including IL-6 [10,24] and IL-8 [17,19,20,24,29,35], thus significantly impacting on the inflammatory process and ultimate degree of tissue injury. Inhibition of p38 with SB 239063 suppressed LPS-induced increased plasma TNF levels and reduced adjuvant arthritis paw inflammation in the Lewis rat [4] and was 3-fold more potent than the first generation inhibitor SB 203580. Although the exact mechanism is not fully understood, both in vitro and in vivo studies have demonstrated that inhibition of p38 MAPK correlates with decreased levels of pro-inflammatory cytokine release [1,25,29,30].

In a recent study, we also demonstrated that protein concentrations for IL-6 and IL-1ra did not significantly increase until 12 h following distal electrocoagulation of the middle cerebral artery [32]. In addition, the increased levels of IL-1β were biphasic increasing at 4 h, approaching baseline, and then significantly increasing again at 12 h. The timing of increased protein levels agrees with the infiltration of inflammatory cells as assessed by myeloperoxidase activity in this particular model. Since cytokine production and neutrophil infiltration can be delayed up to 12 h or may fall below the lower limit of quantification at these time points, p38 MAPK inhibitors may be beneficial via additional/alternative mechanisms.

The rapid phosphorylation of p38 following severe stroke [22] suggests that the activation of this signaling cascade may be, to some degree, independent of the brain inflammatory response. The effects of SB 239063, a novel, second-generation p38 inhibitor, have been evaluated in an in vitro model of oxygen-glucose deprivation induced neuronal cell death [4]. SB 239063 significantly reduced hippocampal CA1 cell death (up to 40%) produced by this in vitro ischemia in cultured organotypic brain slices. These in vitro data suggest that p38 inhibition may directly protect neurons, independent of blood flow effects, in addition to effects at blocking inflammatory cytokine/mediator production. Therefore, the aim of the present study was to examine the temporal profile of p38 MAPK phosphorylation following moderate focal stroke and to determine whether SB 239063 provides very early direct neuronal protection in vivo.

2. Methods

2.1. Middle cerebral artery occlusion

Male spontaneously hypertensive rats (SHR; Taconic Farms, Germantown, NY) weighing 300–350 g, were used in this study. Body temperature was maintained at 37°C during all surgical procedures and during recovery from anesthesia (i.e. until normal locomotor activity returned). Animals were anesthetized with pentobarbital (65 mg/kg, i.p.) and underwent permanent middle cerebral artery occlusion (MCAO) as described previously [5–7]. Briefly, the middle cerebral artery was exposed through a 2–3-mm^2 craniotomy made just rostral to the zygomatic-squamosal skull suture and occlusion of the middle cerebral artery was achieved at the level of the inferior cerebral vein.

2.2. Western analyses for p38 MAPK

For Western blotting experiments, the brain was removed and rapidly dissected into ischemic (I) and non-ischemic control cortices (C) and snap frozen at −80°C. Both cortices were homogenized in 1 ml of standard lysis buffer for every 100 mg of tissue wet weight. Based on protein assay, equivalent amounts of protein (e.g. 30 μg/well) were be loaded onto a 10% Bis Tris Gel from Novex. The gel was run at a constant 130 V for 90 min. The gel was transferred at 100 V for 1 h at room temperature or overnight at 4°C at 23 V to a nitrocellulose membrane (Protran, Schleicher and Schuell BA85, 0.45-μm pore). After the transfer step, the membrane was soaked in blocking solution (ZyMed) for 1 h at room temperature. The membrane was then washed quickly twice and then three times with 1× Tween/DPBS for 10 min each on an orbital shaker. The primary antibody (New England Biolabs kit #9210) was diluted 1:1000 in blocking solution and allowed to incubate overnight at 4°C on slow shake. The membrane was then washed three times as described above. The 2°C HRP-linked antibody (New England Biolabs) was diluted 1:2000 (anti-rabbit HRP) in blocking solution for 1 h at room temperature on a slow shaker. Following incubation with the secondary antibody, the membrane was washed twice quickly followed by two 10-min washes as above. The membrane was then soaked in ECL reagent (Amersham RPN 2106 1:1 ration of reagent 1 and reagent 2) for 1 min (ECL was not mixed more than 15 min prior to use). The membrane was placed in a hypercassette (Amersham RPN 11648) with hyperfilm (Amersham RPN 1674H) and exposed for between 30 s and 1 min to develop the film. Western blots were scanned and densitometries were calculated using NIH 1.62 Image Quant software. Data are represented as a relative fold increase in the amount of p38 MAPK in the ischemic and non-ischemic cortices relative to sham operated tissue on the same side of the brain. This ratio allows for direct comparisons between blots as well as for different exposure times.

2.3. Dose range for oral SB 239063

The dose volume for oral studies using SB 239063 was 10 ml/kg. The vehicle was prepared using a 0.5% tragacanth (Sigma, St. Louis, MO) solution and hydrochlo-

ric acid (pH 3.0–3.5). SB 239063 powder was added to the solution and the pH was maintained at 3.0–3.5 for optimal effectiveness. In order to determine the most effective dose, the animals received a dose of 5, 15, 30, and 60 mg/kg 1 h prior to middle cerebral artery occlusion and a subsequent dose of SB 239063 at 6 h post MCAO.

2.4. Histologic evaluation of infarct size

Rats were euthanized by an overdose of sodium pentobarbital (200 mg/kg, i.p.). The brains were immediately removed and 2-mm coronal sections were cut from the entire forebrain area (i.e. from the olfactory bulbs to the cortical-cerebellar junction), using a brain slicer (Zivic-Miller Laboratories). The coronal sections were immediately stained in a solution of 1% triphenyltetrazolium chloride as described [8]. Sections were transferred to 10% formalin (in 0.1% sodium phosphate buffer) for at least 24 h and then photographed and analyzed as described previously [6,7]. Briefly, brain injury was quantified using an Optimas image analysis system (DataCell) and the degree of brain damage was corrected for the contribution made by brain edema/swelling as described previously [33,38]. Hemispheric swelling and infarct volume (mm^3) were calculated from infarct areas measured from the sequential forebrain sections. Infarct size was expressed as the percent infarcted tissue in reference to the contralateral hemisphere. Slice by slice analysis was also performed to evaluate the extent of agreement between histology and MRI determinations of brain injury.

2.5. Diffusion weighted imaging

The magnet was programmed to acquire both diffusion weighted and T2 weighted images. For magnetic resonance imaging, the animals were intubated for respiratory gating and were subsequently maintained on a mixture of 1.5–3% isoflurane and 0.8 l/min of medical grade air. The core body temperatures of the animals were monitored with a rectal probe and maintained at 37±1°C. Diffusion weighted imaging (DWI) was performed using a full birdcage resonator on a 4.7 T/40 cm Bruker ABX spectrometer equipped with a gradient coil insert as described previously [12]. Briefly, DWI data were collected as follows: SE: TR/TE=1500/45 ms; 128×128; FOV=3×3 cm; slice thickness=2 mm; G=10 G/cm; Δ=25 ms; 4 NEX; δ=10 ms; b-factor=1550 s/mm^2. The animals were placed in the magnet within the 1st hour and a half post surgery and data were acquired at a 2-h time point. Following imaging, the animals were allowed to recover from anesthesia under supervision. All MR images were transferred to an SGI UNIX workstation and were analyzed with the software package ANALYZE™ (CN Software, UK) in exactly the same manner as histologic sections were analyzed. Infarct assessment and hemispheric volume was obtained by manual tracing. The percent of hemispheric infarct was calculated over all slices (Σ the area of lesion per slice/Σ the contralateral area per slice) for each animal using three data sets acquired at 2 h. T2 weighted MR images were acquired at 24 h and 7 days post injury and hemispheric infarct was calculated as described above.

2.6. Assessment of neurologic deficit

Each rat then was evaluated for neurological deficits using two graded scoring systems as previously described [6,7,9]. The neurologic condition of the rat was assessed at 24 h and 7 days after the ischemic insult. The animals were assigned a numerical score ranging from 0 (no observable evidence of neurologic deficit) to 3 (most severely injured) as described by Bederson et al. [9]. In addition to the Bederson test, rats underwent various sensorimotor and proprioception behavioral tests further evaluating forelimb and hindlimb function. Collectively, the Bederson score, hindlimb, and forelimb individual scores were added together. A normal healthy rat would have a global neurologic score of 0. A rat with maximal neurologic deficit would have a global neurologic score of 7.

2.7. Statistics

Absolute measurements (e.g. infarct volume, densitometry) for each individual parameter were analyzed by parametric and non-parametric analyses of variance (ANOVA). Post hoc comparisons among groups were evaluated using Fisher's protected least significant differences (LSD) and Dunnett's test where appropriate. Neurobehavioral scores were evaluated using Kruskal–Wallis median test. Differences among groups were assessed using Mann–Whitney U-test.

3. Results

3.1. Evidence of p38 phosphorylation

To determine whether the activity of p38 MAPK was altered following permanent middle cerebral artery occlusion, ischemic (n=4) and control cortices (n=4) were subjected to Western analysis using p38 and phospho p38 MAPK antibodies. There were no significant changes in non-phosphorylated p38 MAPK levels between sham-operated and MCAO animals from 15 min to 5 days post stroke (figure not shown). Fig. 1 demonstrates a very rapid activation of p38 MAPK. Levels of phosphorylated p38 MAPK were significantly ($P<0.05$) upregulated at both 15 min (2.3-fold) and 1 h (1.8-fold) post injury compared to non-ischemic and sham-operated tissue. The levels returned to baseline by 4 h and there were no significant changes up to 5 days post injury or in sham operated tissue at 15 min, 1, 4, or 24 h.

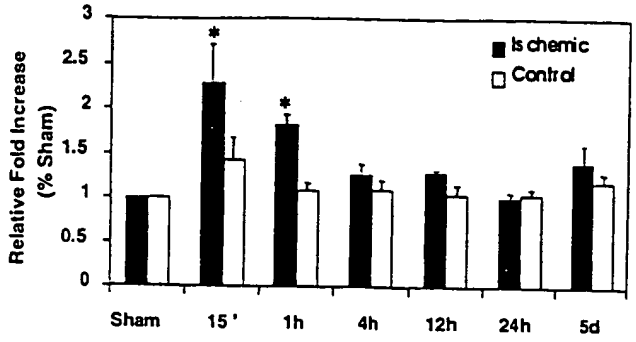

Fig. 1. Changes in phosphorylated p38 MAPK following MCAO in the rat. Ischemic tissue (n=4) and control tissue (n=4) are represented as mean±S.E. The mean for each group is normalized to sham tissue run on the same gel. Comparisons were considered significant if * P<0.05.

3.2. Dose related neuroprotection

In order to further evaluate whether selective p38 inhibition, using a well-characterized p38 inhibitor, is neuroprotective following MCAO, simple behavioral tests to assess neurologic function and triphenyltetrazolium chloride staining for histologic measures were utilized to evaluate several doses of SB 239063 (n=6–10 per group). Control animals received an equal volume of acidified tragacanth (vehicle). SB 239063 provided significant neuroprotection in stroke (Fig. 2). The vehicle treated group exhibited an infarct volume of 148±8 mm^3 and a consistent neurologic grade score of 2.6±0.1. Animals, which received the lowest dose (5 mg/kg) of SB 239063, had significantly (P<0.05) less neurologic deficits as evidenced by a mean neurologic score of 1.9±0.5 compared to the vehicle treated group. This neurologic protection correlated to a 42% (P<0.01) reduction in total infarct volume (86±12 mm^3). The most dramatic neuroprotection, with improvements in behavior, and reductions in infarct size, were observed in the group of animals treated with 15 mg/kg of SB 239063. Animals within this group had a significantly (P<0.01) lower mean neurologic score (1.5±0.3) as compared to the vehicle treated group. The behavioral improvements correlated to a 48% reduction in infarct volume (77±11 mm^3) which was significant (P< 0.01) over vehicle treated animals. A higher dose of SB 239063 (30 mg/kg) was also neuroprotective. At this dose, behavioral deficits were significantly (P<0.05) reduced and infarct volume was reduced (P<0.05) by ~29%. At a dose of 60 mg/kg, SB 239063 did not reduce functional deficits (2.3±0.4) or confer any significant histologic protection (14%). Fig. 3 demonstrates that the optimal dose of SB 239063 (15 mg/kg) provided significant neuroprotection throughout the forebrain. These relationships for total infarct volume were also evident for percent hemispheric infarct as well as infarct measurements that were corrected for swelling.

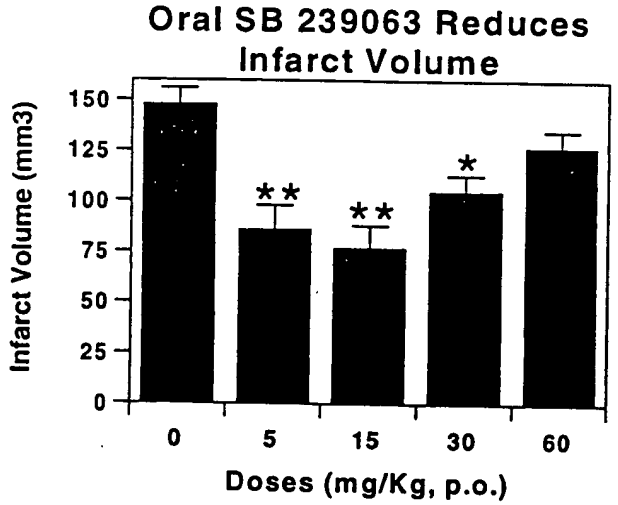

Fig. 2. Dose-related effects of oral SB 239063 on infarct volume following MCAO. Oral administration of SB 239063 at 5, 15, and 30 mg/kg significantly reduced total infarct volume at 24 h following MCAO (n=6–10/group). All data are represented as mean±S.E. Differences were considered significant at * P<0.05 and ** P<0.01 compared with vehicle treated group.

3.3. Development and resolution of infarct size

To better evaluate the neuroprotective effects following p38 inhibition with the most effective oral dose of SB 239063 (15 mg/kg), global neurologic deficit (GND), diffusion weighted imaging (DWI), and T2 weighted MRI were evaluated following MCAO to monitor both the development and resolution of the infarct. DWI was used to measure the early effects of cytotoxic edema to reflect the areas ultimately at risk of irreversible injury. This has been shown previously to reflect ultimate degree of injury/ protection in this model [12]. We have previously demon-

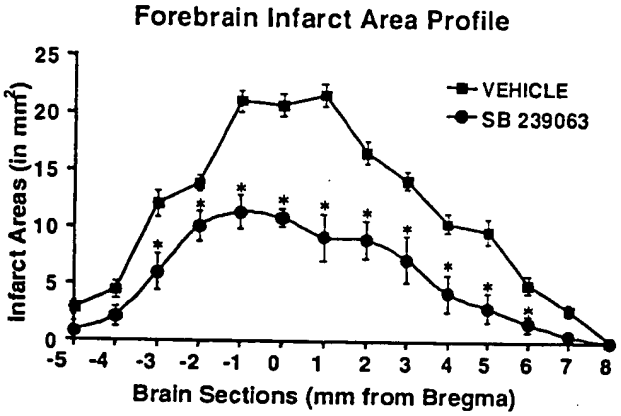

Fig. 3. Effects of SB 239063 (15 mg/kg) on infarct volume over sequential forebrain slices. Brain sections correspond to various distances from the skull landmark bregma for vehicle treated and SB 239063 (15 mg/kg) groups. This figure demonstrates the significant neuroprotection exhibited throughout the forebrain as a result of oral administration of SB 239063 (15 mg/kg). All data are represented as mean±S.E. Differences were considered significant at * P<0.05 compared with vehicle treated group.

strated a very high correlation ($r>0.90$, $P<0.01$) between TTC histology and T2 weighted MRI at 24 h post MCAO. In this particular study, using a separate group of animals treated with either vehicle ($n=5$) or 15 mg/kg SB 239063 ($n=5$), this relationship was reconfirmed and again highly correlative ($r=0.94$, $P<0.01$). At 2 h post injury, there was a significant ($P<0.05$) reduction in the area at risk (e.g. cell depolarization, diffusibility of water) for the SB 239063 (109.7±9.7 mm^3) treated group compared to the vehicle group (160.8±9.3 mm^3). At 24 h post injury, there was ~30% reduction in infarct size between the SB 239063 (146.8±7.8 mm^3) and the vehicle treated group (206.4±11.3 mm^3). There was a good correlation ($r=0.74$, $P<0.01$) between the protection observed using early DWI ($n=9$/group) and 24 h T2 MR imaging ($n=9$/group). At 1 day post MCAO, SB 239063 (15 mg/kg) therefore, provided dramatic neuroprotection which was associated with a significant ($P<0.01$) reduction in neurologic deficit from 4.4±0.4 in the vehicle treated group to 1.6±0.3 in animals receiving SB 239063 (15 mg/kg). At 7 days post MCAO, animals receiving SB 239063 (15 mg/kg) maintained a 30% reduction in infarct size which was significant ($P<0.05$) compared to the vehicle treated group. The neuroprotection that was observed with T2 weighted MRI over time is represented in Fig. 4. The neuroprotection as assessed by infarct size was not attributable to any significant differences in amount of swelling within the injured brain between the treatment groups. By day 7, the ischemic hemisphere had become significantly smaller due to macrophage. Identical results were obtained when the infarct volumes were corrected for swelling. At 7 days post injury, SB 239063 (15 mg/kg) also significantly ($P<0.01$) improved neurologic outcome assessed by a GND of 1.2±0.2 compared to 3.8±0.5 for the vehicle treated group.

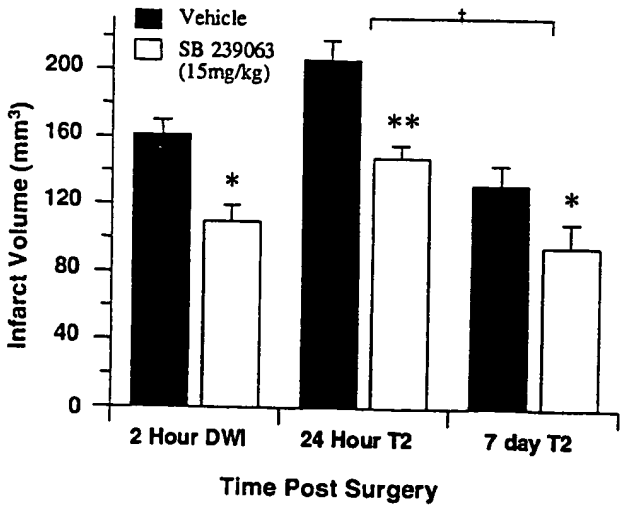

Fig. 4. Effects of SB 239063 (15 mg/kg) on infarct volume over a 7-day period. All data are represented as mean±S.E. Differences were considered significant at * $P<0.05$ compared to vehicle, ** $P<0.01$ compared to vehicle, and † $P<0.05$ compared to day 1.

4. Discussion

4.1. p38 Mitogen activated protein kinases

This was the first study designed to examine the temporal relationship of p38 activation following moderate focal stroke, where this type of ischemia produces a discrete cortical infarct. In the normal brain, p38 (non-phosphorylated) is present in a wide variety of cell types including neurons, astrocytes, endothelial cells and leukocytes. Following permanent middle cerebral artery occlusion, we did not expect or observe any changes in non-phosphorylated p38 MAPK at any of the time points (15 min to 5 days) evaluated. The levels of non-phosphorylated p38 MAPK were nearly identical between both the ischemic and contralateral control hemispheres for injured and sham-operated animals. These results are consistent with an earlier study where Walton et al. reported no change in p38 MAPK levels following bilateral common carotid occlusion [41].

4.2. p38 MAPK and delayed cell death

In the same study, however, following 7 min of global ischemia, gradual changes in activated/phosphorylated p38 MAPK activation were observed over 47 days with the peak appearing at 4 days [41]. This type of stimulus usually leads to apoptotic cell death over the course of 1 week. Bhat et al. have studied this response in more detail using Nissl-stained sections and demonstrated that the loss of CA1 pyramidal neurons was maximal between 3 and 4 days after ischemia. Taken together, these results along with several others suggest that p38 activation may play a role in apoptotic cell death [11,21,27,36,42]. Several studies have evaluated the effects of p38 inhibition following apoptosis [18,21,23,27,34,36,42]. In cultured cerebellar granule neurons, SB 203580 prevented the activation of caspase 3 activity as well as glutamate-induced apoptosis [18,23]. In PC12 pheochromocytoma cells, withdrawal of nerve growth factor induces apoptotic cell death, which is preceded by p38 MAPK activation [27]. Within PC12 cells, apoptosis (programmed cell death) may be regulated by a balance of survival promoting extracellular signal regulating kinases (ERK) and stress-activated protein kinases (SAPK) [42]. SB 203580 abolished this apoptotic cell death and enhanced neuronal survival within this cell culture system [21,42]. However, there are only limited studies evaluating the effects of p38 activation and inhibition following stimuli that result primarily in necrotic cell death [4].

4.3. Activation of the p38 pathway following focal stroke

In the present study, p38 MAPK activity was evaluated following moderate focal stroke where cell death primarily occurs in cortical tissue via necrosis. Following MCAO, there was an early and robust activation of p38 MAPK at 15 min post injury which was maintained at 1 h but then returned to baseline levels by ~4 h post MCAO. These data are consistent with previous data demonstrating that p38 MAPK activation is an early response to the cellular stress (i.e. ischemia, ATP depletion, ionic changes) elicited by severe focal ischemia [4]. The early increases in p38 activation are also in agreement with immunohistochemical changes in p38 MAPK phosphorylation observed in other models of focal stroke. In particular, p38 activity increased early as observed at 1, 3 and 6 h following thread MCAO [4], and was prolonged, remaining elevated for at least 24 h, following transient MCAO [22]. Therefore, the changes in p38 MAPK activation may be differentially regulated in response to either the duration and/or intensity of the stimuli. The rapid activation of p38 post-ischemia may be directly neurodestructive to the neurons rather than inducing a delayed cell death via apoptosis. In this SHR model of moderate focal stroke, the damage is maximal within the first 24 h and primarily restricted to the cortex. In addition, there is little evidence for apoptotic cell death in this model.

Rapid activation of p38 MAPK has also been observed following myocardial/ischemia reperfusion injury [34]. The maximal level of p38 MAPK activation in isolated Langerdorff perfused hearts was at 10 min following reperfusion which is consistent with the results obtained in this study. Pre-treatment with SB 203580, a specific p38 inhibitor, significantly improved post-ischemic cardiac function but treatment initiated after 10 min had no effect. Having established significant activation/phosphorylation of p38 MAPK in the brain up to and including 1 h following moderate focal stroke, the current study was designed to determine whether inhibition of p38 MAPK activation would provide early direct neuroprotection following ischemia.

4.4. Early neuronal protection using SB 239063

SB 239063 is a second generation pyridinyl imidazole compound with potent activity and improved selectivity [4]. Specific p38 MAPK inhibitors have been widely studied in both in vivo and in vitro models. The early rapid activation of p38 in this model suggests that treatment may be most beneficial if initiated prior to or at the time of peak activation. In the present study, SB 239063 (administered 1 h pre- and 6 h post MCAO) provided significant neuroprotection following focal stroke. We have also previously demonstrated that intravenous treatment, with SB 239063, was neuroprotective when initiated within the first 15 min following MCAO [4]. In a separate study intravenous treatment with SB 239063 prevented activated p38 from phosphorylating its downstream targets at plasma levels that provided significant neuroprotection from brain injury [3]. Although we have demonstrated that SB 239063 blocks p38 activity at 1 h and is neuroprotective when administered prior to or at the time of peak activation, we have not fully evaluated the therapeutic window for this class of drug. The most effective dose of SB 239063 (15 mg/kg) was then evaluated using MR imaging to monitor both the development and resolution of the infarct from 2 h to 7 days post MCAO. There was a striking difference in diffusion weighted imaging (DWI) at 2 h post MCAO. The DWI contrast is based on lack of mobility of water in the extra-cellular environment. During the initial phases of ischemic injury, areas where the extracellular space is dramatically reduced (restricted for free water mobility due to cell swelling) are highlighted by this technique [40]. Therefore, it is implied that DWI measures the early effects of cytotoxic edema. Thus, the size of the infarct very early following stroke in essence reflects the areas ultimately at risk of irreversible injury but not necessarily irrevocably injured at that early time point. In fact, a good agreement with post-mortem histology demonstrates that areas at risk, as predicted by DWI early following MCAO in this model, do finally get damaged permanently as measured by TTC staining at 24 h [12]. At 24 h post injury, the animals treated with SB 239063 exhibited a 30% reduction in infarct volume compared to vehicle treated animals. Therefore, the data demonstrate that the protection observed at 2 h is identical to the neuroprotection seen at 24 h. By 7 days post injury, both infarct size and hemispheric size had decreased due to the phagocytic activity of macrophages which creates a cavitation within the necrotic tissue and was probably responsible for the decrease in hemispheric volume [13] but the 30% protection in the drug treated group was maintained. Overall, the data demonstrate that treatment with SB 239063 (15 mg/kg) provides dramatic neuroprotection up to and including 7 days post MCAO. Since the protection observed at 7 days was identical to that assessed by DWI at 2 h, it appears that no additional cell death (e.g. via apoptosis) had occurred over 1 week. The data also suggest that the reduction in DWI hyperintensity observed at 2 h following treatment with SB 239063 may be attributed to a direct protective effect on brain cells. This in vivo study is in agreement with previous findings where SB 239063 (20 μM) significantly reduced hippocampal CA1 cell death (up to 40%) produced by OGD in cultured organotypic brain slices [4]. These in vitro data suggest that p38 inhibition may directly protect neurons, independent of blood flow effects, in addition to effects at blocking inflammatory cytokine/mediator production. We have previously demonstrated that SB 239063 has no cardiovascular or cerebrovascular effects in spontaneously hypertensive rats (SHR) [4]. SHR were chosen because they exhibit a more

consistent degree of brain damage following permanent or transient focal ischemia than do normotensive rats using dorsal electrocoagulation of the MCA [6] because of their limited collateral circulation [14–16]. These early improvements in neuronal injury following p38 inhibition do reflect final outcome up to and including 1 week post injury.

Studies are ongoing to determine the effective therapeutic window for SB 239063 following focal stroke. In addition multiple in vitro cell systems are being utilized to better understand how SB 239063 may be interfering with the early pathophysiological events. These detailed in vitro studies are necessary to fully understand the role of p38 following ischemia and the mechanism(s) by which it can be beneficial.

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